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IDENTIFICATION AND SYNTHESIS OF VOLICITIN AND RELATED COMPONENTS FROM BEET ARMYWORM ORAL SECRETIONS

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Abstract—Oral secretion of beet armyworm caterpillars (BAW), when applied to damaged tissues of corn seedlings, induces the seedlings to emit volatile compounds that attract the natural enemies of the caterpillars. The key elicitor present in BAW oral secretions is *N*-[17-hydroxylinolenoyl]-L-glutamine (volicitin). Analysis of the oral secretion showed that it also contained *N*-[17-hydroxyolinoleoyl]-L-glutamine, free 17-hydroxylinolenic, and 17-hydroxylinoleic acid, the glutamine conjugates of linolenic and linoleic acid as well as free linolenic and linoleic acid. Here we present the identification and synthesis of the hydroxy acids and of glutamine conjugates.

Key Words—*Spodoptera exigua* Hübner, *Zea mays* L., elicitors, isolation, identification, synthesis, volicitin, 17-hydroxylinolenic acid, 17-hydroxylinoleic acid, linolenic acid, linoleic acid, glutamine conjugates.

INTRODUCTION

Injury by insect herbivores induces plants to release a blend of volatile terpenoids and indole that attract natural enemies of the herbivores (Turlings et al., 1990, 1991). This intriguing defensive reaction is triggered when substances

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in the oral secretion of the insect herbivores contact damaged plant tissue. Isolation of the inducing factors in the oral secretion of beet armyworm (*Spodoptera exigua* Hübner) caterpillars resulted in a single fraction that contained all the biological activity of the crude secretion. This fraction contained two components in about equal concentrations (Turlings et al., 2000). Both components had almost identical retention times on HPLC and identical UV spectra; however, only one of them induced corn (*Zea mays* L.) seedlings to release the same blend of volatile compounds as are released when they are damaged by caterpillar feeding (Turlings et al., 2000). The identification and synthesis of the active component, *N*-[17-hydroxylinolenoyl]-L-glutamine, named volicitin, was reported by Alborn et al. (1997). Here we present an improved synthesis of volicitin and the synthesis of the second component, which was identified as *N*-[17-hydroxylinoleoyl]-L-glutamine by mass and infrared spectroscopy and by chemical transformations. We also describe an improved analysis of BAW oral secretion that revealed the presence of related components including free linolenic, linoleic, 17-hydroxylinolenic, and 17-hydroxylinoleic acid, as well as the glutamine conjugates of linolenic and linoleic acid.

METHODS AND MATERIALS

Isolation of Biologically Active Natural Product. Following procedures described in Turlings et al. (1993), 50 ml of oral secretion were collected from third to fifth instar beet armyworms by gentle squeezing, causing them to regurgitate. Each purification step was monitored by analyzing the induced release of terpenoids and indole from corn seedlings (*Zea mays*, variety Ioana and LG11 sweet corn) that had been allowed to take up a test solution through the cut stem overnight (Turlings et al., 1993). The purification is described in detail in Alborn et al. (1997) and Turlings et al. (2000). The crude oral secretion was centrifuged at 1600*g* for 30 min and filtered through a sterilizing membrane (0.22 μm Millex GV, Millipore, Bedford, Massachusetts). An equal amount of 50 mM pH 3 sodium phosphate buffer was added, and the precipitated proteins were removed by centrifugation as before. The active material was purified with a series of water–acetonitrile gradient reverse-phase HPLC separations (LDC 4100 pump, SM 5000 diode array detector, LDC Analytical, Riviera Beach, Florida) with a Nova Pac C_{18} reverse phase column, 4-μm particle size, 4 mm ID $\times$ 150 mm long (Waters, Millford, Massachusetts) and with a reverse-phase ODS-AQ S-5 column, 4.6 mm ID $\times$ 250 mm long (YMC, Kyoto, Japan). The UV absorption was monitored at 200 nm. Two closely related and difficult to separate components were isolated and designated **B** and **C.** Component **B** was as biologically active as the crude oral secretion while **C** showed no activity (Turlings et al., 2000).

Spectrometric Instrumentation. A VG Analytical Zab-spec (Fison Instruments, Manchester, England) high-resolution mass spectrometer was used for fast atom bombardment (FAB) analysis of the natural products. Up to 4 μl of 10 ng/μl solutions in 50% acetonitrile–water were added to a 10-μl glycerol matrix. Samples were protonated by addition of 1 μl trifluoroacetic acid. Sodium adducts were obtained by adding 1 μl of 1 M aqueous sodium chloride. Polyethylene glycol (1 μl) with an average mass of 400 Da (PET 400) was added to the FAB glycerol matrix to give reference ions of known mass for an exact calibration of the mass scale and subsequent estimation of the elemental composition.

Daughter ion FAB-MS/MS spectra were obtained from samples in the same matrix as above, and analyzed on a tandem four sector mass spectrometer (Jeol HX/HX110A, Tokyo, Japan). The nitrogen collision gas was adjusted to give 60% reduction in intensity of the parent ion.

Electron impact (EI) and chemical ionization (CI) mass spectrometric analyses were obtained on a Finnigan TSQ 700, quadrupole spectrometer (Finnigan MAT, San Jose, California) interfaced to a Hewlett-Packard 5890 gas chromatograph (Palo Alto, California), equipped with either a polar OV 351 column (25 m long $\times$ 0.25 mm ID; Scandinaviska Genetec, Kungsbacka, Sweden), or a nonpolar DB-1 column (25 m $\times$ 0.25 mm ID; J&W Scientific, Folsom, California). Methane was used as the reaction gas for CI. Synthesis products were analyzed with a Shimadzu QP-5000, quadrupole GC-MS (Kyoto, Japan), in the EI mode, equipped with an RTX-5 column, (30 m $\times$ 0.25 mm ID, SGE Austin, Texas), or with a Jeol SX102 instrument equipped with an HP-5 column (15 m $\times$ 0.20 mm ID).

GC-FTIR analysis of natural products were performed on a Hewlett-Packard model 5965B Fourier transform infrared spectrometer interfaced to a model 5890 GC equipped with a DB-1 column (25 m long $\times$ 0.32 mm ID; J&W scientific). Synthesis products were analyzed with the same type of instrumentation fitted with an RTX-5 amine column (30 m $\times$ 0.32 mm). FTIR spectra of neat liquids were obtained with a Perkin-Elmer 1600 series FTIR instrument. In all GC analyses injections were made in the splitless mode at 225°C with the column held at 60°C for 3 min, then programmed at 10°C/min to 250°C.

Acid Methanolysis. Following procedures modified from Mee et al. (1977), we evaporated an amount of each purified compound equivalent to 100 μl oral secretion to dryness and added 50 μl of dry methanol and 10 μl acetic anhydride. In a sealed 1-ml glass ampule filled with nitrogen, the sample was then heated to 100°C for 10 min, allowed to cool, and evaporated to dryness with a gentle stream of N_2. Fifty microliters of CH_2Cl_2 were added, and the sample was analyzed by GC-MS.

Location of Double Bonds and Hydroxyl Groups. Following procedures in Attygalle et al. (1995), we evaporated 50-μl oral secretion equivalents of each fatty acid methyl ester obtained by methanolysis (above) to dryness and redis-

solved in 10 μl ethanol. After the addition of 30 μl of 10% hydrazine in ethanol and 30 μl of 0.6% hydrogen peroxide in ethanol, the solution was heated at 60°C for 1 hr. The sample was cooled to room temperature and 35 μl of 5% aqueous HCl was added. The solution was extracted with two 40-μl aliquots of GC[2] Hexane (Burdick and Jackson, Muskegon, Michigan), and the hexane solution was washed with four 50-μl aliquots of water. The partially reduced hydroxy acid methyl esters were ozonized following standard procedures (Beroza and Bierl, 1966, 1967) and the products analyzed by GC-MS to determine the locations of the double bonds.

To determine the location of the hydroxyl groups, the double bonds of the methyl esters were reduced by adding 5 mg of PdO to 50 μl of oral secretion equivalent sample solution in 50 μl of ethyl acetate, and H_2 was gently bubbled through the solution for 18 hr. The products were analyzed by GC-MS.

Pyrrolidide derivatives of the fatty acid methyl esters also were prepared by dissolving a sample in 10 μl of 1% glacial acetic acid in freshly distilled pyrrolidine and heating to 100°C for 30 min in a sealed tube (Anderson, 1978). The product was cooled to room temperature, 10 μl of CH_2Cl_2 were added, and the product analyzed by GC-MS. This derivative significantly reduces the characteristic hydrocarbon MS fragmentation pattern of long chain fatty acids and was originally developed to determine the location of double bonds in the chain. It also resulted in increased intensity of diagnostic ions for the location of hydroxyl groups.

HPLC Analysis of BAW Oral Secretion. Following determination of the structure of volicitin, we developed an HPLC method to analyze the acidic components of the BAW oral secretion. To 1 ml of filter sterilized oral secretion 100 μl acetic acid and 2 ml of CH_2Cl_2 were added. The solution was shaken for 5 min, and the organic phase was evaporated to dryness under vacuum. One milliliter of 50 mM pH 8 sodium phosphate buffer was added and 10 μl of the solution was analyzed on HPLC with the ODS-AQ S-5 column described above (flow, 1 ml/min, gradient 40 to 100% of solvent B in A in 10 min, 100% B for 15 min, A = 0.05% aqueous acetic acid and B = 0.05% acetic acid in acetonitrile). The column eluent was monitored by UV detection at 200 nm.

Synthesis of 17-Hydroxylinolenic Acid (Figure 1). Under an argon atmosphere, 1 M solution of ethylmagnesium bromide in 65 ml tetrahydrofuran (THF) was added dropwise to 10.7 g (59 mmol) of the ethoxyethyl ether of 3,6-heptadiyn-1-ol (Brandsma, 1988; Huang et al., 1983) in 20 ml of THF at 0°C. After 1 hr, 4 ml of freshly distilled acetaldehyde in 10 ml of THF were added dropwise, and the mixture was stirred for 1 hr. Five milliliters of freshly distilled pyridine and 7.6 ml of benzoyl chloride were then added, and after 3 hr, 20 ml of saturated aqueous NH_4Cl were added, and the mixture was extracted with ether (3 × 50 ml). The combined extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was chromatographed on silica gel

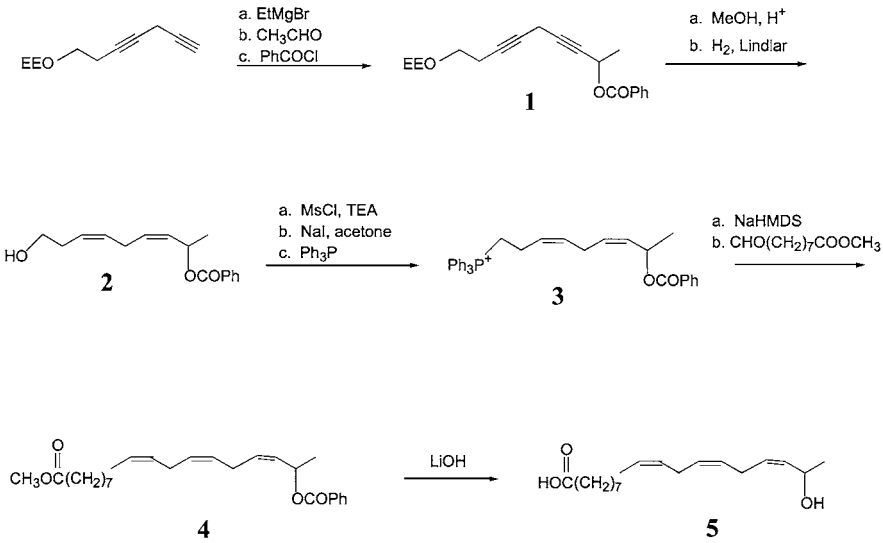

FIG. 1. Synthetic scheme for the synthesis of 17-hydroxylinolenic acid.

(100 g; hexane eluent) to provide 19.0 g of **1** [(8-benzoyloxy)-3,6-nonadiyn-1-ol ethoxyethyl ether, >95% pure by GC analysis]. GC-FT-IR: 3074, 2981, 2943, 2890, 1735(s), 1602, 1453, 1382, 1357, 1310, 1271(s), 1176, 1106, 1025, 891, and 857 cm^{-1}; MS, *m/z* (rel %) 255 (3), 115 (10), 105 (85), 91 (12), 77 (25), 73 (100), 51 (10), and 45 (80). HR-MS, calc. for $C_{20}H_{24}O_4$: 328.1675; observed, 328.1674 and calc. for $C_{19}H_{21}O_4$ (M-CH_3): 313.1440: observed 313.1450.

A solution of 18.2 g of **1** and 100 mg of *p*-toluenesulfonic acid in 100 ml of MeOH was stirred for 2 hr. GC-MS analysis indicated that the ethoxyethyl ether group had been removed to provide the corresponding alcohol. After the addition of 5 ml of saturated $NaHCO_3$, the solvent was removed in vacuo. The residue was taken up in ether and dried over anhydrous $MgSO_4$ and the solvent removed to provide 13 g of crude diyne alcohol. A solution containing 8.0 g of this alcohol and 0.6 ml of quinoline in 100 ml ethanol was hydrogenated over 2.0 g of Lindlar catalyst (Aldrich Chemical Co.). As the uptake of H_2 slowed, additional portions of catalyst were added (total of 6.0 g). After the uptake of 1450 ml of hydrogen, the mixture was filtered through Celite (Fisher Chemical Co.) and the solvent was removed in vacuo. The residue was taken up in ether, washed with dilute HCl and saturated $NaHCO_3$, and dried over $MgSO_4$. Filtration and removal of the solvent provided 7.8 g of **2** [(*Z,Z*)-(8-benzoyloxy)-3,6-nonadien-1-ol, >90% pure by GC analysis, less than 1% of other double bond isomers]. GC-FT-IR, 3667, 3023, 2964, 2938, 1733(s), 1452, 1314, 1267(s), 1178, 1102, 1046, 905, and 859 cm^{-1}; MS, *m/z* (rel %), 260 (1, M+), 229 (2), 175 (3), 138 (12), 123 (4),

105 (100), 93 (16), 91 (14), 79 (45), and 77 (55); HR-MS, calc. for $C_{16}H_{20}O_3$: 260.1412; observed, 260.1407, and calc. for $C_{15}H_{17}O_5$ (M-CH_2OH): 229.1229; observed, 229.1231.

A solution of 6.0 g (23 mmol) of **2** and 2 ml of triethylamine in 50 ml of CH_2Cl_2 was cooled and treated with 2 ml of methanesulfonyl chloride. After 5 hr the mixture was diluted with 200 ml diethyl ether and washed sequentially with dilute HCl, water, and saturated $NaHCO_3$. The organic solution was dried over anhydrous $MgSO_4$ and after filtration the solvent was removed to provide the unstable mesylate. This product was immediately dissolved in 50 ml of dry acetone containing 5.7 g of NaI and refluxed under Ar for 1.5 hr. The solution was cooled, and diluted with 100 ml of diethylether, and filtered through 60 g of neutral alumina with excess diethyl ether. Concentration in vacuo provided the pale yellow iodide. Analysis by GC-MS of the extremely unstable mesylate and iodide derivatives showed that they were ca. 90% pure. The iodide was immediately taken up in 70 ml acetonitrile containing 5.25 g of triphenylphosphine and refluxed 6 hr. Most of the solvent was removed in vacuo and the residue was triturated with diethylether. The ether layer was decanted and the residue was washed with 2 × 50 ml of ether. The solvent was removed from the residue to provide 8.0 g of the phosphonium salt **3** as a brittle foam. A 1 M solution of sodium hexamethyldisilazide in hexane (14 ml) was added dropwise to 8 g (12.7 mmol) of **3** in 50 ml of THF under Ar at −78°C. After 15 min, 2.45 g of methyl 9-oxononanoate (Pennington et al., 1953; Burgstahler et al., 1976) in 5 ml of THF were added dropwise, and the resulting mixture was stirred at −78°C for 1 hr and warmed to room temperature for 8 hr. After addition of 10 ml sat aq. NH_4Cl and 30 ml diethyl ether, the organic phase was separated, dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. The residue was triturated with 70 ml of 1 : 1 ether–petroleum ether. After filtration and removal of the solvent, the organic layer provided 1.3 g of residue that was approximately a 1 : 1 mixture (by GC-MS) of triphenylphosphine oxide and **4** (methyl 17-benzoyloxylinolenate); MS, *m/z* (rel %) 290 (7, M-$PhCO_2H$), 175 (2), 161 (2), 147 (5), 133 (14), 119 (16), 105 (100), 94 (20), 93 (16), 91 (28), 81 (11), 80 (28), 79 (34), 77 (27), 67 (20), 55 (28), 41 (30); HR-MS, calc. for $C_{19}H_{30}O_2$ (M-$PhCO_2H$), 290.2246; observed, 290.2246. The mixture was dissolved in 20 ml THF and cooled to −20°C, which resulted in a slow precipitation of the triphenylphosphine oxide over several days, which then was removed by filtration. The solvent was removed by a gentle stream of N_2. The protecting group was removed by stirring a 500-μg aliquot of neat material with 2 ml of 1 M LiOH overnight at room temperature under N_2. The solution was extracted 3× with 3 ml CH_2Cl_2 and 3× with 3 ml hexane. The pH of the water solution was then adjusted to <7 with 1 M HCl and the product extracted three times with 2 ml of CH_2Cl_2. While gently heating and stirring the combined solution, we added approximately 2 ml of hexane, and the solution was then allowed to cool, result-

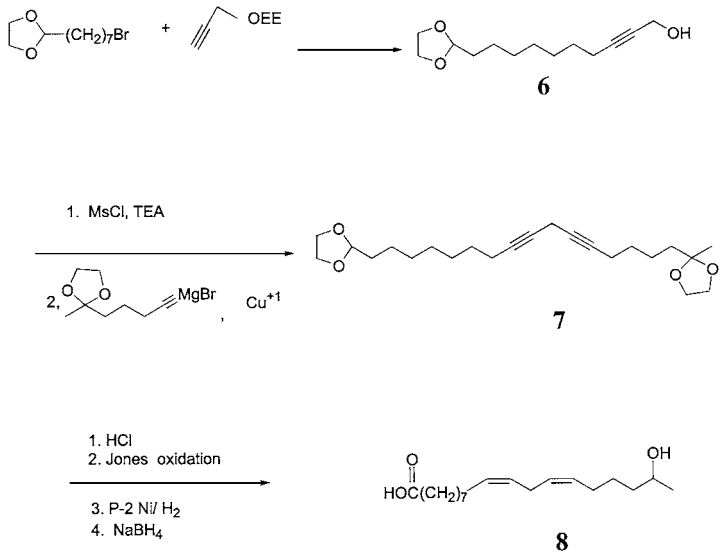

FIG. 2. Synthetic scheme for the synthesis of 17-hydroxylinoleic acid.

ing in precipitation of most of the benzoic acid. The final solution was concentrated under vacuum to thick yellow liquid. HPLC analysis with UV detection at 200 nM showed two major components, benzoic acid (45% of total area) and hydroxylinolenic acid (50% of total area). GC analysis after treatment with 4 M HCl in MeOH showed the hydroxy acid to be 95% pure, with less than 1% of *trans* isomers and approximately 3% of benzoic acid.

Synthesis of 17-Hydroxylinoleic Acid (Figure 2). Under Ar, 1.6 M solution of *n*-butyllithium in hexane (65 ml) was added dropwise to a solution containing 5.1 g (40 mmol) of the ethoxyethyl ether of propargyl alcohol in 20 ml THF at 0°C. After 30 min, 8.79 g (35 mmol) of freshly distilled 2-(7-bromohept-1-yl)-1,3,-dioxolane (Gil et al., 1996) were added dropwise followed by 15 ml of dimethylsulfoxide (DMSO). The mixture was warmed to room temperature over 8 hr. After addition of 150 ml hexane, the mixture was washed four times with 50 ml of water, dried over anhydrous $MgSO_4$, and after filtration the solvent was removed in vacuo. The residue was taken up in 100 ml of benzene containing 5 ml of ethylene glycol and 100 mg *p*-toluenesulfonic acid and heated to reflux for 2 hr to selectively remove the ethoxyethyl protecting group. The cooled mixture was washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated in vacuo and the residue was chromatographed over silica gel (100 g; hexane/ether 9 : 1 eluent) to provide 5.2 g (66%) of **6** [2-(10-hydroxy-8-decyn-1-yl)-1,3-dioxolane]. GC-FTIR: 2939, 2873, 1397, 1139, 1014, and 943

cm^{-1}; MS, *m/z* (rel %) 225 (1), 209 (1), 195 (1), 73 (100), 55 (12), and 45 (50). HR-MS, calc. for $C_{23}H_{21}O_3$ (M-1): 225.1491; observed, 225.1498.

Compound **7** {2[16-(1,3-dioxolan-2-yl)-4,7,-hexadecadiyn-1-yl]-2methyl-1,3-dioxolane} was prepared by dropwise addition of 12.6 g (60 mmol) of 2-(3-bromoprop-1-yl)-2-methyl-1,3-dioxolane (Bellas et al., 1969) to a suspension of 8.3 g of lithium acetylide–ethylene diamine complex in 20 ml of THF, followed by the addition of 15 ml of DMPU. The mixture was stirred overnight and worked up in the usual manner to provide 6.9 g of 2-(4-pentyl-1-yl)-2-methyl-1,3-dioxolane (74% yield), bp 77–82°C (22 mm Hg). GC-FTIR: 3328, 2989, 2958, 2885, 2118, 1447, 1378, 1307, 1245, 1217, 1132, 1063, 943 and 883 cm^{-1}; MS, *m/z* (rel %) 153 (1), 139 (25), 99 (20), 87 (100), 55 (10), and 43 (90); HR-MS calc. for $C_9H_{14}O_2$: 154.0994; observed, 154.0994.

Methanesulfonyl chloride (0.5 ml) was added to a solution containing 1.18 g (5.22 mmol) of **6** and 0.6 ml of triethylamine in 15 ml of CH_2Cl_2 cooled to 0°C. The mixture was stirred 4 hr (completion of reaction confirmed by TLC), washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated in vacuo. Analysis of the product showed one major component (the unstable mesylate of **6**): MS, *m/z* (rel %) 303 (1, M-1), 225 (1, M-$MeSO_2$), 207 (1), 73 (100, dioxolanyl). This product was passed through a short silica gel column, eluted with hexane–diethyl ether (2 : 1), and the solvent was removed in vacuo.

A solution containing 1.23 g (8 mmol) of 2-(4-pentyn-1-yl)-2-methyl-1,3-dioxolane in 10 ml of THF was treated with 9 ml of 1 M ethylmagnesium bromide in THF. After 1.5 hr the mixture was cooled to 0°C and 85 mg of CuCl were added. After 20 min, the above mesylate in 3 ml of THF was added dropwise, and the mixture was stirred overnight and allowed to warm to room temperature. The mixture was diluted with diethyl ether, and washed sequentially with saturated NH_4Cl and brine, dried over $MgSO_4$, and filtered. After removal of the solvent, the residue contained only the ethylene ketal of 6-heptyn-2-one and the dine **7** (70%). MS, *m/z* (rel %) 362 (1, M^+), 347 (4), 99 (10), 87 (100), 73 (83), 55 (12), and 45 (11); HR-MS scale, calc. for $C_{22}H_{34}O_4$: 362.2457; observed, 362.2444.

The above mixture was taken up in 10 ml of acetone containing 2.0 ml of 5% aqueous HCl at room temperature. After 6 hr the reaction was neutralized with saturated $NaHCO_3$ and the solvent removed in vacuo. The mixture was extracted three times with ether and the combined ether extracts were dried ($MgSO_4$), filtered, and the solvent was removed in vacuo. The residue was taken up in 40 ml of acetone and added dropwise to a cold (0°C) solution of 1.5 g of CrO_3 in 15 ml of 3.5 M H_2SO_4. After 5 hr, the usual work up (Oehlslager et al., 1986) provided 0.6 g of 17-oxo-9,12-octadecadiynoic acid (40% from **6**). Methyl ester (CH_2N_2) GC-FT-IR: 2939, 2865, 1754, 1439, 1360, 1313, 1167 and 1022 cm^{-1}; MS, *m/z* (rel %) 303 (M-1, 1), 289 (2), 273 (1), 162 (8), 161

(10), 147 (10), 145 (10), 131 (11), 119 (16), 117 (18), 105 (23), 91 (25), 87 (2), 81 (9), 79 (16), 74 (6), 55 (20), and 43 (100); HR-MS, calc. for $C_{19}H_{28}O_3$: 304.2038; observed, 304.2046.

A solution of the 17-oxo-9,12-octadecadiynoic acid in 5 ml of ethanol was added to P-2 nickel freshly prepared from 0.75 g of nickel(II) acetate under a hydrogen atmosphere. After 4 hr, the usual work up (Millar and Oehlschlager, 1984) provided (*Z,Z*)-17-oxo-9,12-octadecadienoic acid. Methyl ester (CH_2N_2) GC-FTIR: 3016, 2935, 2865, 1756, 1440, 1360, 1170 and 1023 cm^{-1}; MS, *m/z* (rel %) 308(1, M^+), 377 (2), 376 (3), 250 (5), 150 (6), 135 (7), 133 (6), 121 (8), 108 (15), 107 (16), 95 (12), 94 (20), 93 (21), 91 (14), 87 (10), 81 (22), 80 (32), 79 (42), 74 (7), 67 (41), 55 (25), 43 (100), and 41 (38); HR-MS, calc. for $C_{19}H_{32}O_3$: 308.2351; observed, 308.2361.

The (*Z,Z*)-17-oxo-9,12-octadecadienoic acid prepared above was reduced with $NaBH_4$ as described in the literature (Oehlschlager et al., 1986) to provide 0.35 g of 17-hydroxylinoleic acid (**8**) (58% yield from 17-oxo-9,12-octadecadiynoic acid). Methyl ester (CH_2N_2) GC-FTIR: 3016, 2935, 2865, 1758, 1441, 1358, 1241, and 1173 cm^{-1}; MS, *m/z* (rel %) 292 (1, M-18), 250 (2), 178 (5), 164 (5), 163 (4), 150 (7), 149 (8), 135 (16), 122 (12), 121 (16), 101 (10), 108 (25), 107 (24), 95 (32), 94 (50), 93 (30), 91 (15), 87 (15), 81 (39), 80 (37), 79 (61), 74 (14), 67 (64), 59 (100), 55 (50), 45 (12), 43 (30), and 41 (65); HR-MS, calc. for $C_{19}H_{34}O_3$: 310.2508; observed, 310.2512.

Synthesis of Glutamine Conjugates of 17-Hydroxylinolenic and 17-Hydroxylinoleic Acids. Glutamine conjugates of the hydroxyacids were prepared by procedures modified after Sheehan et al. (1965). Five hundred milligrams of L-glutamine-*p*-nitrobenzyl ester (Bachem Bioscience Inc. King of Prussia, PA) were dissolved in 3 ml of dimethylformamide (DMF), and then 200 μl of water and 250 μl of triethylamine (TEA) were added. In a second flask, 400 mg of the hydroxyacid was dissolved in 3 ml DMF and then 500 mg of 1-hydroxybenzotriazole (HOBT) (Sigma) were added and allowed to totally dissolve. Subsequently 300 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) (Sigma) were added. After stirring for 2 min, the contents of the first flask were added to the second and the mixture was stirred overnight at ambient temperature under N_2. The following day, 5 ml of CH_2Cl_2 were added, and the mixture extracted with 5 ml of 100 mM aqueous ammonia, 5 ml of water, 5 ml of 100 mM acetic acid, and finally three additional 5-ml aliquots of water before the CH_2Cl_2 fraction was concentrated under vacuum to a thick yellow liquid (approximately 300 mg). HPLC analysis revealed for each coupling one major component [*N*-(17 hydroxylinolenoyl)-*p*-nitrobenzyl-glutamine and *N*-(17 hydroxylinoleoyl) *p*-nitrobenzyl-glutamine].

The nitrobenzyl ester was dissolved in 2 ml THF, 2 ml of 1 M Na_2S in 50% water–THF was added, and the mixture stirred over night to remove the protective *p*-nitrobenzyl group (Lammert et al., 1978). The mixture was con-

centrated to near dryness and redissolved in 5 ml water. The aqueous solution was extracted with 4 × 5 ml CH_2Cl_2 to remove most of the *p*-nitrobenzyl alcohol. HPLC analysis of the aqueous fraction revealed a totally deprotected final product (70%) and the presence of a closely eluting by-product (25%) with identical UV absorption. There were also traces of *p*-nitrobenzyl alcohol (5%). The crude product in water was chromatographed on a solid-phase extraction column (Mega bondelut C_{18}, Varian, Harbor City, California, 25 mm ID × 25 mm long). The column was rinsed with 40 ml water to remove sulfide. Twenty milliliters of 20% acetonitrile–water removed remaining traces of *p*-nitrobenzoyl alcohol. The partially purified product was recovered with 40 ml of 60% acetonitrile–water followed by 20 ml of 100% acetonitrile (200 mg). HPLC analysis by the improved procedures for analysis of BAW oral secretion described above revealed the fraction to contain only the expected product and the by-product (70/30%). Pure conjugates were obtained by HPLC on the YMC ODS-AQ column eluted with 45% acetonitrile and 0.5% acetic acid in water at a flow of 1.0 ml/min, monitored by UV detection at 200 nm.

Synthesis of Glutamine Conjugates of Linolenic and Linoleic Acids. L-Glutamine *t*-butyl ester was coupled to 500 mg of linolenic and linoleic acids (Aldrich, Milwaukee, Wisconsin) by the same procedures as described above. The protective *t*-butyl group was removed by dissolving and stirring the product overnight in 2 ml of 88% formic acid. Five milliliters of CH_2Cl_2 was then added and the solution was extracted three times with 5 ml of water. The organic phase was concentrated to dryness under vacuum to give 600 mg of the product (>95% pure). The main impurity was noncoupled free fatty acid.

RESULTS

HPLC separations of BAW oral secretion monitored by the corn seedling bioassay described in Turlings et al. (1993) showed consistently that all activity appeared to be located in one fraction that contained two very similar components (Turlings et al., 2000). However, a final separation revealed that only one of these components, **B**, identified as *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin) had biological activity. Fraction **C** was identified by the same mass spectrometric and micro-degradative analysis as described for volicitin (fraction **B**) in Alborn et al. (1997). Fast atom bombardment mass spectroscopy (FAB-MS) analysis gave exact mass *m/z* 425.3050 $(M+H)^+$ in the positive ion mode and subsequently a molecular weight of 424.297 Da for the neutral molecule. There were also traces of *m/z* 407 $(M+H\text{-}H_2O)^+$. This component was not analyzed in the negative ion mode. The addition of sodium chloride to the FAB matrix gave the same result as for **B**, with reduced intensity of the M+1 ion at *m/s* 425 and subsequent strong *m/z* 447 $(MH+Na)^+$ and 469 $(MNa+Na)^+$. These

results indicated that the molecular weights of **B** and **C** only differed by the mass of two hydrogens.

FAB-MS/MS daughter ions of the M+1 ions at m/z 423 for **B** and m/z 425 for **C** gave very strong ions representing the loss of water [**B**: m/z 405 (423 – 18), **C**: m/z 407 (425 – 18)]. The lower mass region of both components showed characteristic hydrocarbon fragmentation combined with an identical pattern of m/z 147, 130, 101, 84, 67, which resembled the electron impact (EI) mass spectra of glutamine (NIST, 1995). A daughter ion spectrum of pure glutamine (M+1 = 147) strongly supported the presence of glutamine by showing the same characteristic fragments (m/z 147, 130, 101, 84, 67). Subtraction of glutamine, linked via an ester or amide bond would give $C_{18}H_{30}O_3$ as the elemental composition of the second part of component **B** and similarly $C_{18}H_{32}O_3$ for **C**.

GC analysis of both components after acid methanolysis showed two prominent peaks. Chemical ionization (CI) MS analysis of the first of thees peaks, common to both components, revealed a prominent $(M+1)^+$ ion at m/z 144 and electron impact (EI) MS analysis revealed a molecular ion at m/z 143 and diagnostic ions at m/z 84 (base peak), 56 and 41, identifying it as the methyl ester of pyroglutamate (NIST, 1995), which by congruence in GC retention time and mass spectrum with the product of glutamine treated in the same way confirmed the presence of glutamine in both components. The CI mass spectrum of the second GC peak for **B** showed no molecular ion but a predominant fragment at m/z 291 due to a loss of water $(M+1-18)^+$. Loss of methanol gave an ion at m/z 277 $(M+1-32)^+$, and the loss of both water and methanol gave an ion at m/z 259 $(M+1-18-32)^+$. The EI spectrum of the same peak showed a very weak molecular ion at m/z 309 and a strong m/z 290 due to the loss of water $(M-18)^+$. The lower mass region of the spectrum also showed a characteristic fragmentation pattern of ions (14 and 12 Da apart), strongly suggesting a straight-chain unsaturated hydrocarbon. These results were consistent with the methyl ester of an 18-carbon hydroxy acid. The GC trace from **C** showed a second peak with a slightly longer GC retention time than the second peak from **B**. The CI mass spectrum of this peak showed a dominating m/z 293 (M+1–18) and no molecular ion. Loss of methanol gave an ion at m/z 279 $(M+1-32)^+$, and the loss of both water and a methanol gave an ion at m/z 261 (M+1–18). The EI spectra showed a smaller m/z 292 and no molecular ion but otherwise a fragmentation very comparable to that of **B**.

Both methyl esters were reduced by treatment with PdO/H_2 over night. GC-MS (CI) analysis showed an identical product with a dominating ion at m/z 296 (M+1–18). These results indicated that component **B** and **C** contained an unsaturated 18-carbon hydroxy acid with three and two double bonds, respectively, and that both contained glutamine.

Fourier transform infrared analysis of the two hydroxy acid methyl esters produced diagnostic absorption at 3646 cm^{-1}, confirming a hydroxyl group and

absorption bands at 3019, 2935, 2865 cm^{-1} and indicating straight-chain nonconjugated unsaturated hydrocarbons. The intensity of the 3019 cm^{-1} peak relative to the others indicated that **B** contained one more double bond than **C** and the absence of a peak in the area of 980 cm^{-1} indicated that no *trans* double bonds were present. Both molecules showed intense absorptions at 1758 cm^{-1} as expected for methyl esters.

These results confirmed that both molecules consisted of two subunits, glutamine and a C_{18} hydroxyacid with two or three *cis* double bonds. These two components could be attached in three ways to give the right elemental composition. However, only an amide bond between glutamine and the acid moiety of the hydroxy acid would result in a free hydroxyl as indicated by the FAB-MS experiments, without also giving a free amine, for which there was no evidence.

The methyl esters of the hydroxy C_{18} acids were subjected to further microdegradative analysis to determine the positions of the double bonds and the hydroxyl group. Partial reduction resulted in both cases in a mixture of mono- and diunsaturated products as established by GC-MS analysis. The mixtures were then ozonized and CI-GC-MS analysis showed the presence of three diagnostic GC peaks for **B** with $(M+1)^+$ ions at m/z 187, 229, and 271, corresponding to $H(CO)(CH_2)_nCOOCH_3$ with $n = 7$, 10, and 13, respectively. Methyl linolenate treated in the same way gave identical products. Thus, the olefinic bonds in the chain are located on carbons 9, 12, and 15, and the alcohol group on either the 17th or 18th carbon. CI-GC-MS analysis of the **C** sample showed the presence of two diagnostic GC peaks with $(M+1)^+$ ions at m/z 187 and 229, corresponding to $HCO(CH_2)_nCOOCH_3$ with $n = 7$ and 10, respectively. Thus the olefinic bonds were located on carbons 9 and 12, and **C** was confirmed as containing hydroxylated linoleic acid but with the position of the hydroxyl still unclear.

EI-GC-MS analysis of the reduced methyl esters showed the identical product with no visible molecular ion but a strong ion at m/z 296 (60) $(M+1-18)$. Weak m/z 299 (5) $(M-15)^+$ and strong m/z 270 (100)/271 (35) $(M-44)^+$ was indicative of a hydroxyl group on C-17. EI mass spectra of a pyrrolidide derivative of the reduced products produced the expected diagnostic ions at m/z 309 (20) $(M-44)^+$ and m/z 338 (30) $(M-15)+$ confirming the C-17 location of the hydroxyl group.

These data proved that the components isolated from the regurgitant of BAW larvae were *N*-(17-hydroxylinolenoyl)-L-glutamine, which we named volicitin (component **B**), and *N*-(17-hydroxylinoleoyl)-L-glutamine (component **C**). We could find no previous reports of these compounds in the literature.

Analysis of Oral Secretion. After the structure of volicitin was determined, a new method was developed for analysis of volicitin and similar compounds in oral secretions of BAW and other insects. Reverse-phase HPLC analysis of the methylene chloride extract of acidified BAW oral secretion revealed several related acidic components with similar UV absorption (Figure 3). The most polar

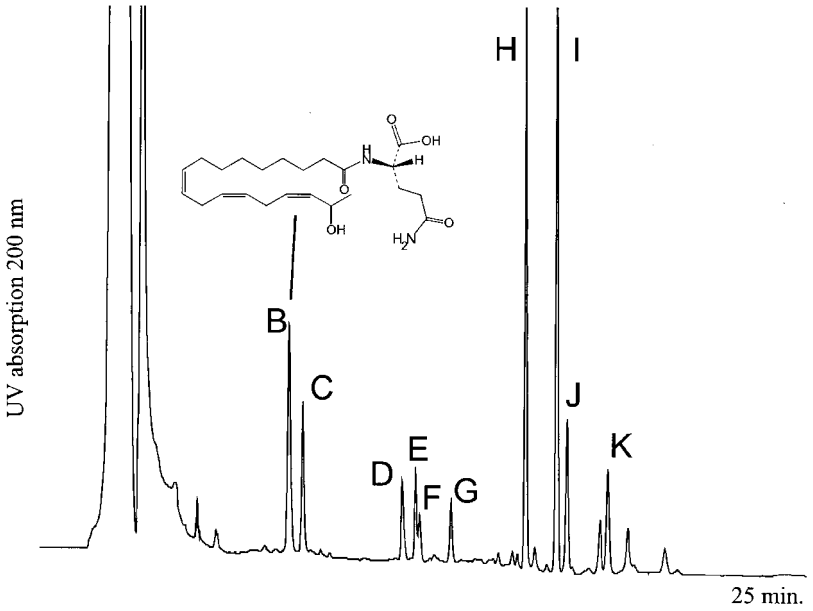

FIG. 3. HPLC separation of acidic components in BAW oral secretion. The YMC ODS-AQ S-5 column (4.6 mm ID × 250 mm long) was eluted at a flow of 1 ml/min with a gradient 40–100% solvent B in A in 10 min and then 100% B for 15 min. A = 0.05% aqueous acid, B = 0.05% acetic acid in acetonitrile. The column eluent was monitored by UV detection at 200 nm. **B**: Volicitin, (*N*-[17-hydroxylinolenoyl]-L-glutamine), **C**: *N*-[17-hydroxylinoleoyl]-L-glutamine, **D**: 17-hydroxylinolenic acid, **E**: *N*-linolenoyl-L-glutamine, **F**: 17-hydroxylinoleic acid, **G**: *N*-linoleoyl-L-glutamine, **H**: linolenic acid, **I**: linoleic acid, **J**: unknown unsaturated C_{18} acid, **K**: oleic acid.

components were volicitin (**B**) and **C**. The presence of free 17-hydroxylinolenic and 17-hydroxylinoleic acids (peaks **D** and **F**) was confirmed by GC-MS analysis of their methyl esters and by coinjection of synthetic components on HPLC and GC. The free linolenic and linoleic acid were identified as major components by coinjection of commercial standards (peaks **H** and **I**) and by GC-MS analysis of the methyl esters of the collected peaks and of the standards. Two components with retention times similar to the free hydroxy acids (peaks **E** and **G**), and UV absorption identical to that of volicitin, were collected and analyzed. FAB-MS gave a molecular weight of 406 Da for **E** and 408 Da for **G**. GC-MS analysis after acid methanolysis gave in both cases a peak corresponding to pyroglutamate methyl ester. Peak **E** also gave a methyl linolenate and **G** gave methyl linoleate. This suggested that these two components were *N*-linolenoyl-L-glutamine (**E**) and *N*-linoleoyl-L-glutamine (**G**).

Synthesis of Glutamine Conjugates. We have earlier shown that only the *N*-[17-hydroxylinolenoyl]-L-glutamine (volicitin) was biologically active in eliciting volatile production and that the D-glutamine form had no biological activity (Alborn et al., 1997). We therefore chose to use only L-glutamine in developing an improved synthesis of volicitin and in the synthesis of component **C.** The earlier described procedures (Alborn et al., 1997) resulted in less than 10% final product.

Racemic 17-hydroxylinolenic acid (**5**) and 17-hydroxylinoleic acid (**8**) were synthesized by the routes illustrated in Figures 1 and 2. The synthesis of **5** was based on the lithium salt-free Wittig method to form the final *Z* double bond (Bestmann et al., 1976, 1987), based on standard methodology to prepare the required aldehyde ester and (*Z*,*Z*)-dienephosphonium salt. The synthesis of **8** also was based on previously described methodology (Oelschlager et al., 1986) wherein the preparation of a (*Z*,*Z*)-dienoic acid containing a secondary alcohol includes oxidation of a ketoaldehyde to the keto acid, reduction of a diyne with P-2 nickel to the (*Z*,*Z*)-diene, and subsequent reduction of the ketone to a secondary alcohol.

Both **5** and **8** were coupled with *p*-nitrobenzyl ester of L-glutamine by a method developed for peptide synthesis. This resulted in a significantly higher efficiency than earlier (approximately 75% compared to 10%), but required subsequent deprotection and purification steps. HPLC analysis of the de-protected product showed the presence of an unidentified substance that comprised about 25% of the mixture. This impurity could not be removed by recrystallization or flash chromatography and had to be removed by HPLC. Thus, it still seriously limited the amount of material that could be produced. An alternative protecting group, *t*-butyl ester, could not be used when glutamine was coupled with the hydroxy acids. The necessary acid deprotecting conditions caused the alcohol to shift position on the fatty acid. However, following the procedures above, this protecting group could be used when L-glutamine was coupled with linolenic and linoleic acid and resulted in products that were considerably purer than when the *p*-nitrobenzyl group was used. The synthetic *N*-linolenoyl-L-glutamine and *N*-linoleoyl-L-glutamine coeluted on HPLC with component **E** and **G** (Figure 3). All the synthetic conjugates showed separation characteristics and UV spectra identical to the natural products. FAB-MS analysis gave molecular ions identical to those of the natural products. GC-MS and GC-FTIR analysis of the products after acid methanolysis also produced identical results when compared to methanolysis of the natural products.

DISCUSSION

The structure of volicitin shows an intriguing similarity to substrates of the lipoxygenase pathway (Farmer and Ryan, 1992a; Krumm et al., 1995). In

plant systems studied thus far, biosynthesis and release of volatile compounds appear to be induced by jasmonic acid, which is produced from linolenic acid by the octadecanoid signaling pathway (Krumm et al., 1995). Jasmonates also have been reported to stimulate numerous physiological and defensive processes in plants (Farmer and Ryan, 1992a,b; Krumm et al., 1995). Furthermore, amino acid conjugates of jasmonic acid were suggested to be involved in physiological and developmental processes in many plants (Kramell et al., 1995). Therefore, the presence of an elicitor that is an octadecatrienoate conjugated to an amino acid suggests that the elicitor molecule in some way interacts with or amplifies the octadecanoid pathway in herbivore-damaged plants. This association with the lipoxygenase pathway and jasmonic acid is further strengthened by the fact that the inactive compound **C** is based on linoleic acid, which is not a jasmonic acid precursor.

We have established that the biological activity of BAW oral secretion is not diet related and thus volicitin is not plant produced (Turlings et al., 1993). However, both linolenic and linoleic acids are plant-produced essential fatty acids for Lepidoptera and are regularly added to artificial diet for these insects. We have recently shown (Paré et al., 1998) that beet armyworm larvae synthesize volicitin by hydroxylating linolenic acid obtained from the plant and conjugate the hydroxylinolenic acid to glutamine.

Of the linolenic and linoleic acid related components present in BAW oral secretion, only volicitin induced production of volatiles in corn seedlings in bioassays, and volicitin was shown to be as active as the crude oral secretion. We now know that *N*-linolenoyl-L-glutamine also can induce corn seedlings in a similar way but that it is considerably less active than volicitin (about 30%, Alborn unpublished data), which, combined with the relatively low concentration of this component in the oral secretion, explains why only volicitin was isolated initially. We do not yet know how widespread or uniform the response of plants to this type of molecule will be and if other plants might respond differently to some of the other components found in the BAW regurgitant. We have found that both corn and cotton respond to BAW damage and to the oral secretions of BAW applied to damaged leaves (Paré and Tumlinson, 1997). While some compounds, such as indole, ocimene, and farnesene are released by both plants, others are unique to each plant, undoubtedly as a result of differences in plant chemistry. In addition, both plants respond systemically to BAW oral secretion by releasing induced volatiles from undamaged leaves of injured plants Röse et al., 1996; Turlings and Tumlinson, 1992). Furthermore, it was shown recently that in cotton the induced volatile compounds are synthesized de novo, rather than being released from storage (Paré and Tumlinson, 1997, 1998).

It is possible that different species of herbivorous insects may produce different types of compounds that induce plants to release volatiles. The oral secretions of at least five species of lepidopterous larvae and of grasshoppers

induce corn seedlings to release the same volatile components (Turlings et al., 1993). However, corn seedlings release greater quantities of volatiles when treated with grasshopper oral secretion than when treated with caterpillar secretion (Turlings et al., 1993). Furthermore, we found that feeding damage from *Heliothis virescens* induces the release of different ratios of volatile compounds than does feeding damage by *Helicoverpa zea* on cotton, corn, and tobacco plants (De Moraes et al., 1998). At this point, it seems reasonable to speculate that closely related insect species may have plant volatile elicitors with the same or very similar structures as volicitin and the other components found in beet armyworm, but that these components might not have the same effect on all plants. We also do not know what effect the isolated components may have on other defense systems in plants, such as the systemic induction of anti feedants. For example, beet armyworm feeding induces a strong systemic reaction in cotton plants that results in feeding deterrence and toxicity (Alborn et al., 1996; McAuslane et al., 1997; McAuslane and Alborn, 1998).

It is still unknown what benefit insects gain from these compounds that elicit strong defensive reactions in plants. One possibility is that they are in some way related to or involved in the insects' defense against bacteria and other pathogens. As noted by Farmer and Ryan (1992a), the octadecanoid signaling pathway in plants is similar in many ways, particularly in the chemical structures of the active molecules, to the eicosanoid pathways in animals that produce prostaglandins and leukotrienes. In insects it has been reported that eicosanoids may be involved in mediating cellular responses to bacterial infections as well as regulating several other physiological functions (Stanley-Samuelson, 1994a,b). The production of volicitin and its related components also might be a way for the insect to improve the uptake of two essential fatty acids from the diet. Both volicitin and **C** are much more water soluble than linolenic and linoleic acid, and the attached glutamine might improve absorption and transport by the digestive system.

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REFERENCES

ALBORN, H. T., ROSE, U. S. R., and MCAUSLANE, H. J. 1996. Systemic induction of feeding deterrents in cotton plants by feeding of *Spodoptera* spp. larvae. *J. Chem. Ecol.* 22:919–932.

ALBORN, H. T., TURLINGS, T. C. J., JONES, T. H., STENHAGEN, G., LOUGHRIN, J. H., and TUMLINSON,

J. H. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 276:945–949.

ANDERSON, B. Å. 1978. Mass spectrometry of fatty acid pyrrolidides. *Prog. Chem. Fats Other Lipids* 16:279–308.

ATTYGALLE, A. B., JHAM, G. N., SVATOS, A., FRIGHETTO, R. T. S., MEINWALD, J., VILELA, E. F., FERRARA, F. A., and UCHOAFERNANDES, M. A. 1995. Microscale, random reduction—application to the characterization of (*E*,*Z*,*Z*)-3,8,11-tetradecatrienyl acetate, a new lepidopteran sex-pheromone. *Tetrahedron Lett.* 36:5471–5474.

BELLAS, T. E., BROWNLEE, R. G., and SILVERSTEIN, R. M. 1969. Synthesis of brevicomin, principal sex attractant in the frass of the female western pine beetle. *Tetrahedron* 25:5149–5153.

BEROZA, M., and BIERL, B. A. 1966. Apparatus for ozonolysis of microgram to milligram amount of compound. *Anal. Chem.* 38:1976–1977.

BEROZA, M., and BIERL, B. A. 1967. Rapid determination of olefinic position in organic compounds in microgram range by ozonolysis and gas chromatography. *Anal. Chem.* 39:1131–1135.

BESTMANN, H. J., STRANSKY, W., and VOSTROWSKY, O. 1976. Darstellung lithiumsalzfreier Ylidlosungen mit Natrium-bis(trimethylsily)amid als Base. *Chem. Ber.* 109:1694–1700.

BESTMANN, H. J., ROTH, K., MICHAELIS, K., VOSTROWSKY, O., SHAFER, H. J., and MICHAELIS, R. 1987. Synthese Methylen-unterbrochener Lepidopteren-Polyenpheromone und Strukturanloger. *Liebigs Ann. Chem.* 1987:417–422.

BURGSTAHLER, A. W., WEIGEL, L. O., and SCHAEFER, C. G. 1976. Improved modification of the Rosenmund reduction. *Synthesis* 1976:767–768.

BRANDSMA, L. 1988. Preparative Acetylenic Chemistry. Elsevier, Amsterdam, p. 225.

DE MORAES, C. M., LEWIS, W. J., PARÉ, P. W., ALBORN, H. T., and TUMLINSON, J. H. 1988. Herbivore-infested plants selectively attract parasitoids. *Nature* 393:570–573.

FARMER, E. E., and RYAN, C. A. 1992a. Octadecanoid-derived signals in plants. *Trends Cell Biol.* 2:236–241.

FARMER, E. E., and RYAN, C. A. 1992b. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase-inhibitors. *Plant Cell* 4:129–134.

GIL, S., LAZARO, M. A., MESTRES, R., and PARRA, M. 1996. Sex pheromone of *Chilo supressalis:* Efficient synthesis of *Z*-13-octadenenal and *Z*-9-hexadecenal. *Synth. Commun.* 26:2329–2340.

HUANG, W., PULASKI, S. P., and MEINWALD, J. 1983. Synthesis of highly unsaturated insect pheromones: (*Z*,*Z*,*Z*)-1,3,6,9-Heneicosatetraene and (*Z*,*Z*,*Z*)-1,3,6,9-nonadecatetraene. *J. Org. Chem.* 48:2270–2274.

KRAMELL, R., ATZORN, R., SCHNEIDER, G., MIERSCH, O., BRUCKNER, C., SCHMIDT, J., SEMBDNER, G., and PARTHIER, B. 1995. Occurrence and identification of jasmonic acid and its amino-acid conjugates induced by osmotic-stress in barley leaf tissue. *J. Plant Growth Regul.* 14:29–36.

KRUMM, T., BANDEMER, K., and BOLAND, W. 1995. Induction of volatile biosynthesis in the Lima bean (*Phaseolus lunatus*) by leucine and isoleucine conjugates of 1-oxo- and 1-hydroxyindan-4-carboxylic acid: Evidence for amino acid conjugates of jasmonic acid as intermediates in the octadecanoid signalling pathway. *FEBS Lett.* 377:523–529.

LAMMERT, S. R., ELLIS, A. I., CHAUVETTE, R. R., and KUKOLJA, S. 1978. Azetidinone antibiotics. 19. A simple method for the removal of p-nitrobenzyl acid protective group. *J. Org. Chem.* 43:1243–1244.

MCAUSLANE, H. J., and ALBORN, H. T. 1998. Systemic induction of allelochemicals in glanded and glandless isogenic cotton by *Spodoptera exigua* feeding. *J. Chem. Ecol.* 24:399–416.

MCAUSLANE, H. J., ALBORN, H. T., and TOTH, J. P. 1997. Systemic induction of terpenoid aldehydes in cotton pigment glands by feeding of larval *Spodoptera exigua. J. Chem. Ecol.* 23:2861–2879.

MEE, J. M. L., KORTH, J., and HALPERN, B. 1977. Rapid and quantitative blood amino acid analysis by chemical ionization mass spectrometry. *Biomed. Mass Spectrom.* 4:178–181.

MILLAR, J. G., and OEHLSCHLAGER, A. C. 1984. Synthesis of *Z,Z,Z*-skipped diene macrolide pheromones for *Cryptolestes* and *Oryzaephilus* grain beetles. *J. Org. Chem.* 49:2332–2338.

NIST (National Institute of Standards and Technology). 1995. Mass spectral library on CD-rom, version 1.0, Gaithersburg, MD.

OEHLSLAGER, A. C., CZYZEWSKA, E., AKSELA, R., and PIERCE, H. D. 1986. Improved synthesis of hydroxy acid precursors of macrolide pheromones of cucujid grain beetles. *Can. J. Chem.* 64:1407–1413.

PARÉ, P. W., and TUMLINSON, J. H. 1997. Induced synthesis of plant volatiles. *Nature* 385:30–31.

PARÉ, P. W., and TUMLINSON, J. H. 1998. Cotton volatiles synthesized and released distal to the site of insect damage. *Phytochemistry* 47:521–526.

PARÉ, P. W., ALBORN, H. T., and TUMLINSON, J. H. 1998. Concerted biosynthesis of an insect elicitor of plant volatiles. *Proc. Natl. Acad. Sci. U.S.A.* 95:13971–13975.

PENNINGTON, F. C., CLEMER, W. D., MCLAMORE, W. M., BOGERT, V. V., and SOLOMONS, I. A. 1953. Microbiologically active 4-thiazolidones. *J. Am. Chem. Soc.* 75:109–115.

RÖSE, U. S. R., MANUKIAN, A., HEATH, R. R., and TUMLINSON, J. H. 1996. Volatile semiochemicals released from undamaged cotton leaves—a systemic response of living plants to caterpillar damage. *Plant Physiol.* 111:487–495.

SHEEHAN, J. C., PRESTON, J., and CRUICKSHANK, P. A. 1965. A rapid synthesis of oligopeptide derivatives without isolation of intermediates. *J. Am. Chem. Soc.* 87:2492–2493.

STANLEY-SAMUELSON, D. W. 1994a. Asessing the significance of prostaglandins and other eicosanoids in insect physiology. *J. Insect Physiol.* 40:3–11.

STANLEY-SAMUELSON, D. W. 1994b. Prostaglandines and related eicosanoids in insects. *Adv. Insect Physiol.* 24:115–212.

TURLINGS, T. C. J., and TUMLINSON, J. H. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proc. Natl. Acad. Sci. U.S.A.* 89:8399–8402.

TURLINGS, T. C. J., TUMLINSON, J. H., and LEWIS, W. J. 1990. Exploitation of herbivore-induced plant odors by host seeking parasitic wasps. *Science* 250:1251–1253.

TURLINGS, T. C. J., TUMLINSON, J. H., HEATH, R. R., PROVEAUX, A. T., and DOOLITTLE, R. E. 1991. Isolation and identification of allelochemicals that attract the larval parasitoid *Cotesia marginiventris* to the micro habitat of one of its hosts. *J. Chem. Ecol.* 17:2235–2251.

TURLINGS, T. C. J., MCCALL, P. J., ALBORN, H. T., and TUMLINSON, J. H. 1993. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *J. Chem. Ecol.* 19:411–425.

TURLINGS, T. C. J., ALBORN, H. T., LOUGHRIN, J. H., and TUMLINSON, J. H. 2000. Volicitin, an elicitor of maize volatiles in the oral secretion of *Spodoptera exigua:* Its isolation and bioactivity. *J. Chem. Ecol.* 26:189–202.